

Applicant : Wei-Yu Lo et al.  
Serial No. : 09/778,516  
Filed : February 7, 2001  
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Attorney's Docket No.: 12875-002001 / 0643-5299US

REMARKS

Applicants hereby submit that the enclosures fulfill the requirements under 37 C.F.R. §1.821-1.825. The amendments in the specification merely insert the paper copy of the Sequence Listing and sequence identifiers in the specification. I hereby state, as required by 37 C.F.R. §1.821(g), that the enclosed submission includes no new matter.


Attached hereto is a marked-up version of the changes made to the specification by the current amendment.

Please apply any charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: \_\_\_\_\_

7-9-01

  
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**"Version With Markings to Show Changes Made"**

In the specification:

Paragraph beginning at page 4, line 21, has been amended as follows:

FIG. 4 is a diagram showing the constructs of the pCLP7 and pCLP8. Nucleotides 687 to 735 of SEQ ID NO:1 and nucleotides 736 to 784 of SEQ ID NO:1 are shown below.

Paragraph beginning at page 14, line 19, has been amended as follows:

The  $\beta$ -galactosidase gene was amplified from chromosomal DNA of *Lactobacillus delbrueckii* (subsp. *bulgaricus*) by polymerase chain reaction (PCR). The PCR amplification consisted of 0.075 units *Pfu Turbo*<sup>TM</sup> DNA polymerase (STRATAGENE<sup>®</sup>, La Jolla, CA), 1  $\mu$ M each of forward (5'-aagctcatgaTTGGCAGCCAGTCTCCGGGC-3'; SEQ ID NO:3) and reverse primers (5'-gacctcatgaACCGTCGCTAGCGACACGCC-3'; SEQ ID NO:4). PCR was carried out in 4 stages: (i) 95°C for 5 min; (ii) 94°C for 30 sec, 54°C for 30 sec, 72°C for 3 min, x 30 cycles; (iii) 72°C for 10 min; and (iv) hold at 4°C. The amplified DNA product was estimated by 0.8% agarose electrophoresis and ethidium bromide stain, followed with purification by GENECLAN III kit (Bio 101, La Jolla, CA). The purified 3 kb  $\beta$ -galactosidase DNA fragment was ligated into *EcoRV* site of pcDNA3 vector (INVITROGENE). The ligation mixture was transformed into *E. coli* strain DH5 $\alpha$ . The blue-color clones containing the plasmid bearing  $\beta$ -galactosidase gene were selected from X-gal/Amp LB agar plate.

Paragraph beginning at page 15, line 12, has been amended as follows:

The plasmid pVA838 obtained from CCRC (Hsinchu, Taiwan) was used as template for cloning of (Em'P) DNA fragment via PCR. The PCR amplification consisted of 0.075 units *Pfu Turbo*<sup>TM</sup> DNA polymerase (STRATAGENE<sup>®</sup>), 1  $\mu$ M each of forward (5'-TTAACGATCGTTAGAAGCAAACCTTAAGAGTG-3'; SEQ ID NO:5) and reverse primers (5'-TTAACGATCGATGTAATCACTCCTTCT-3'; SEQ ID NO:6). PCR was carried out in 4 stages: (i) 95°C for 5 min; (ii) 94°C for 30 sec, 50°C for 30 sec, 72°C for 30 sec, x 30 cycles; (iii) 72°C for 10 min; and (iv) hold at 4°C. The amplified DNA product was estimated by 1%

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agarose electrophoresis and ethidium bromide stain, followed with purification by phenol/chloroform extraction and ethanol precipitation. The purified 120 bp Em<sup>r</sup>P DNA fragment was ligated into the pCRII vector (INVITROGENE). These clones bearing the pCRII/Em<sup>r</sup>P plasmid were selected from X-gal/Amp LB agar plate as white colonies and further checked by PCR and restriction enzyme analysis.